Triphenodioxazine-1,8-dicarboxylic Acid as an Oxidation Product of 3-Hydroxyanthranilic Acid

Larry A. Hick, Michael K. Manthey and Roger J. W. Truscott*

Department of Chemistry, University of Wollongong,
P.O. Box 1144, Wollongong, 2500, Australia

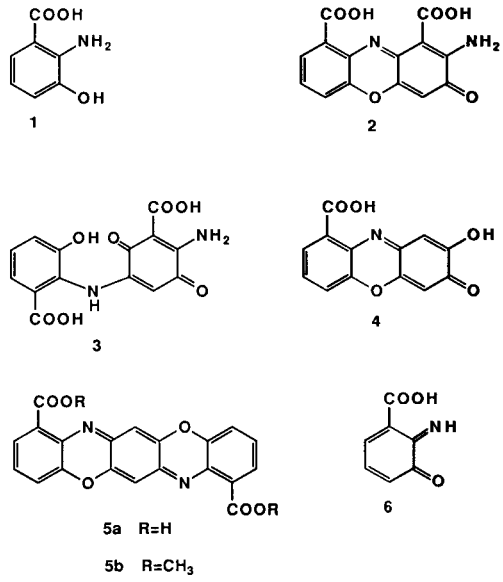
Received February 22, 1989

Revised April 17, 1991

The oxidation of 3-hydroxyanthranilic acid (3OHA) **1** by aqueous buffered potassium ferricyanide produces a number of coloured compounds. These include cinnabarinic acid **2** (yellow), a *p*-quinone dimer **3** (red) and 9-carboxy-2-hydroxy-3*H*-phenoxazin-3-one **4** (brown). Also present by tlc is a bright pink compound. The structure of this compound has been demonstrated to be triphenodioxazine-1,8-dicarboxylic acid **5a**.

J. Heterocyclic Chem., **28**, 1157 (1991).

3-Hydroxyanthranilic acid (3OHA) **1** is a metabolite of the amino acid tryptophan. In aqueous solution 3OHA readily autoxidises to yield cinnabarinic acid **2** [1] and a *p*-benzoquinone dimer **3** [2]. The relative amount of the two compounds formed, is determined by the *p*H of the solution [2,3]. Since we are interested in the oxidation of 3OHA, and in particular the mechanism of reaction of the aminophenol oxidation products with proteins, we are studying the various pathways of oxidation of this compound. Here the results of an investigation into the oxidation of 3OHA by potassium ferricyanide at neutral *p*H are reported.



Results and Discussion.

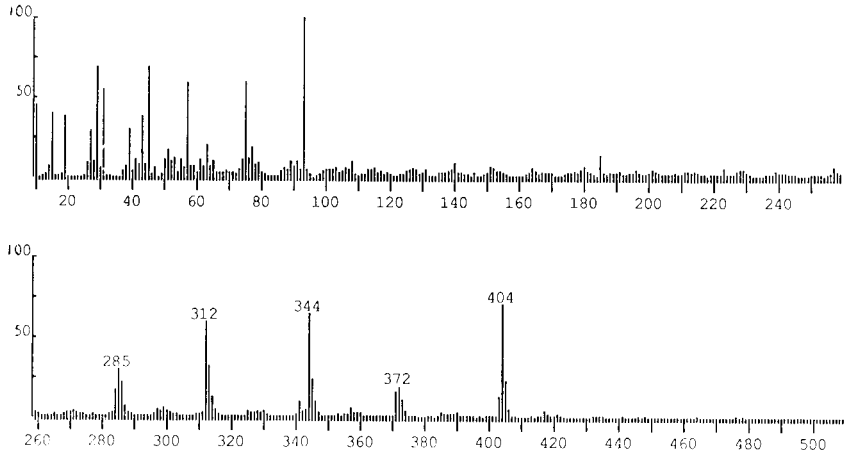
The oxidation of 3OHA with potassium ferricyanide at neutral *p*H gives rise to a variety of products. Many of these are highly coloured. A number of the compounds were identified by a comparison of the mobilities on tlc, colour and uv/visible spectra following elution from tlc plates with standards prepared by oxidation of 3OHA under defined conditions.

Amongst these were cinnabarinic acid **2** (R_f = 0.59), the recently characterised *p*-quinone dimer **3** (R_f = 0.82) [2] and 2-hydroxy-3*H*-phenoxazin-3-one **4** (R_f = 0.50), denoted as Dye V by Butenandt *et al.* [4]. Some 3OHA remained unoxidised. Other minor compounds were not investigated further.

One oxidation product was notable for its brilliant pink colour on tlc (R_f = 0.77). In an attempt to increase the yield of this compound several different oxidation methods were employed. The best was found to be reaction of 3OHA with 1,4-benzoquinone in glacial acetic acid. After incubation at 80-90°, a dark precipitate was formed which was found by tlc to be largely the triphenodioxazine **5a**. A similar result was obtained using 2,5-dihydroxy-1,4-benzoquinone in place of 1,4-benzoquinone.

The product was found to be insoluble in almost all solvents tested, and only sparingly soluble in others (*e.g.* pyridine). Some material could also be dissolved in dilute ammonia solution from where it could be reprecipitated by adjusting the *p*H below 4.

The pink compound could be extracted from ethyl acetate into aqueous sodium carbonate solution, forming a yellow solution. On acidification below *p*H 6, compound **5a** partitioned into the organic phase as a dark pink solution. The uv/visible spectrum also altered dramatically as a function of *p*H; peaks at 513 and 481.5 nm (*p*H 9) being replaced by peaks at 604 and 453 nm (*p*H 4). The presence of the triphenodioxazine nucleus in **5a** was confirmed by running a uv/visible spectrum in 30% ethanol/70% sulfuric acid as described by Schafer *et al.* [5]. The solution turned dark blue and displayed a strong absorbance at 605 nm. Compound **5a** also turned dark blue when dissolved in trifluoroacetic acid. This reaction, as described by Schafer [5], could also be employed as a tlc staining technique. The pink band of compound **5a** derived either from potassium ferricyanide oxidation of 3OHA or condensation of 3OHA with benzoquinone turned intense



1,8-Dicarbomethoxytriphenodioxazine

Table 1

^{13}C NMR Data and Assignmets for **5b** (90MHz, δ (ppm))

C-1,8	C-2,9	C-3,10	C-4,11	C-6,13	C-4a,11a	C-5a,12a	C-6a,3a	C7a,14a	*C*OOCH$_3$	COO*C*H$_3$
130.68	125.71	128.44	118.39	106.84	144.38	147.50	152.33	133.85	166.91	52.48

blue following spraying with methanolic 6*M* hydrogen chloride.

The presence of a triphenodioxazine chromophore indicated by the long wavelength uv absorption was also supported by the mass spectral data. In positive ion FAB ms, an ion at m/z 376 was obtained; in negative ion FAB ms, an ion at m/z 374. High resolution ms on the m/z 376 ion confirmed the molecular formula of **5a** to be $C_{20}H_{10}N_2O_6$ suggesting a dicarboxytriphenodioxazine [6].

Triphenodioxazine itself was synthesized as described by Bolognese *et al.* [6]. In mass spectrometry it displayed similar behaviour to that of compound **5a**: positive ion FAB ms m/z 288; negative ion FAB ms m/z 286.

Attempts to crystallise **5a** were unsuccessful, presumably due to a combination of its extreme insolubility and the presence of minor quantities of polymeric material which proved impossible to remove. Attempted high temperature (>300°) vacuum sublimation of compound **5a** lead to the formation of triphenodioxazine.

Consequently, characterisation of **5a** was performed on the dimethyl ester derivative **5b**, (prepared from **5a** using ethereal diazomethane). Compound **5b** displayed an analogous uv profile to **5a**; the yellow solution of **5b** exhibiting long wavelength maxima at 484.4 nm and 516.6 nm under neutral conditions, whereas acidification resulted in a marked bathochromic shift to new maxima centered upon 583.6 nm and 629.6 nm. In the process the solution of **5b** became blue/violet in colouration. The ^1H nmr spectrum of **5b** indicated the presence of two chemically equivalent methyl ester moieties which occurred as a sharp singlet (δ 3.98). Also indicated were two equivalent olefinic protons (δ 6.60), and aromatic resonances characteristic of a triphenodioxazine ring system substituted in the 1 and 8 positions by methoxycarbonyl moieties [7]. The proton decoupled ^{13}C nmr spectrum of **5b** included resonances characteristic of imino (δ 152.33), enol ether (δ 147.50), and olefinic (δ 106.84) functionalities, the latter of which occurred as a doublet in the off resonance ^{13}C nmr spectrum. Complete ^{13}C nmr spectral data and assignments for **5b** are shown in Table 1, and are based on those reported for 2-amino-3*H*-phenoxazin-3-one [8].

The mechanistic pathway for the formation of the *p*-quinone dimer **3** from the autoxidation of 3OHA has recently been elucidated, and shown to proceed exclusively *via* coupling of molecular oxygen to a phenoxy radical of 3OHA [9]. A similar mechanism is proposed to account for the formation of **3** from the ferricyanide oxidation - the phenoxy radical produced (either autoxidatively or chemically) now reacting with the ambient concentrations of molecular oxygen present in solution. Indeed, when oxidation was carried out by potassium ferricyanide in a solution purged with nitrogen, **3** could not be detected.

Base induced decomposition of **2** or **3**, possibly *via* initial conjugate addition of water/(hydroxyl ion) upon the C-2 position of either **2** of **3** followed elimination of ammonia and a facile decarboxylation from the resulting *o*-quinone intermediate [10] afforded **4**. However, such a mechanism does not seem to be operative in the potassium

ferricyanide oxidation of 3OHA as evidenced by the stability of **2** and **3** towards the oxidant. An alternative mechanism involving hydrolysis or oxidation of the initially formed *o*-quinonimine intermediate **6** derived from the oxidation of 3OHA [9], to the corresponding *o*-quinone, followed by decarboxylation [10] may account for the formation of **4**.

The structure of **5a** is clearly consistent with the known condensation reactions of aminophenols with *p*-quinones in acetic acid [5,7]. Since neither *p*-benzoquinone nor 2,5-dihydroxy-1,4-benzoquinone were able to be detected in the potassium ferricyanide oxidation mixture, an alternative mechanism such as the condensation of two molecules of **1** with the decarboxylated *o*-quinone intermediate derived from **6** may be operative for the formation of the triphenodioxazine **5a** in aqueous solution.

The pink triphenodioxazine thus joins the list of novel coloured compounds derived by oxidation of this naturally occurring aminophenol.

EXPERIMENTAL

The ^{1}H nmr spectra were recorded on Jeol 90 MHz and Jeol 400 MHz spectrometers. Microanalyses were performed by the Microanalytical unit at the Australian National University, Canberra Australia. Fast Atom Bombardment mass spectra (FAB ms) were obtained on a VG Analytical MM12-12 mass spectrometer using an atom beam produced in an Ion Tech Ltd. (UK) saddle field ion source at 8KV and 2mA. The sample was dissolved in a 1:1 mixture of glycerol and concentrated ammonia solution for **5a** or concentrated sulfuric acid for **5b**. Ultraviolet/visible spectra were recorded on a Shimadzu UV-160 spectrophotometer. Tlc was performed on 0.25 mm silica F-254 plates (Merck). Column chromatography was carried out employing silica gel (0.063-0.2 mm, Merck) as the adsorbent.

Oxidation of 3OHA with Potassium Ferricyanide.

3OHA (1.00 g, 6.5 mmoles) was dissolved in 1000 ml of 25 m*M* potassium phosphate buffer *p*H 7. Potassium ferricyanide (6.46 g, 19.5 mmoles) in 100 ml of water was added slowly with vigorous stirring. The mixture was kept at room temperature for 2 hours then adjusted to *p*H 4 with tartaric acid. The brown precipitate was filtered and the filtrate extracted with ethylacetate (3 x 400 ml) and concentrated by rotary evaporation to approximately 100 ml. Portions of the extract were applied to silica tlc plates and developed in butanol/acetic acid/water (12/3/5).

Cinnabarinic Acid (**2**).

Cinnabarinic acid was synthesized as described by Nicholls and Rienits [11]. 3OHA (1 g) was dissolved with warming in 600 ml of ethanol. Recrystallised benzoquinone (1 g) was added and the solution stirred for 1 hour at room temperature. The red precipitate of cinnabarinic acid was removed by filtration and washed with 95% ethanol, yield 364 mg (37%).

6-Amino-3-[2′-carboxy-6′-hydroxyphenylamino]-2,5-dioxo-1,3-cyclohexadiene-1-carboxylic Acid (**3**).

Carbon dioxide-free air was bubbled through a solution of 3OHA (200 mg) in 50 ml of 0.1*M* sodium phosphate buffer *p*H 11.7 for 24 hours. The resulting deep red solution was acidified to *p*H 2.5 with concentrated hydrochloric acid and the precipitate of **3** collected by filtration, yield 108 mg (50%). This material was identical with that prepared previously [2].

2-Hydroxy-3*H*-phenoxazin-3-one (**4**).

Compound **4** was synthesized from **3** as described by Butenandt *et al.* [4]. Compound **3** (160 mg) was dissolved in 40 ml of 1*M* sodium hydroxide at room temperature for 8 hours. The solution was adjusted to *p*H 2.5 with 2*M* hydrochloric acid and left to stand overnight. The precipitate was filtered and washed with 1 m*M* hydrochloric acid, yield 61 mg (47%). Alkaline digestion of **4** [4] yielded the expected products 2,5-dihydroxy-1,4-benzoquinone and 3OHA.

Dicarboxytriphenodioxazine-1,8-dicarboxylic Acid (**5a**).

2,5-Dihydroxy-1,4-benzoquinone (0.50 g) and 3OHA (0.50 g) were added to 100 ml of glacial acetic acid. The mixture was stirred at 80° for 5 hours and the precipitated **5** removed by filtration from the hot solution and washed with acetic acid, yield 376 mg (31%), dec >300°. Crude material was dissolved in a minimum volume of ammonium hydroxide and reprecipitated with dropwise addition of acetic acid. Due to the marked insolubility of the compound we were unable to obtain material of sufficient purity for microanalysis.

HRFAB ms [M+2] calcd. for $C_{20}H_{10}N_2O_6+2H$: 376.0692. Found: 376.0715.

1,8-Dicarbomethoxytriphenodioxazine (**5b**).

To a suspension of **5a** (60 mg) in methanol (10 ml) was added an excess of etheral diazomethane and reaction allowed to continue for no less than 36 hours. Solvent was removed by evaporation, and the residue purified by column chromatography on silica employing chloroform as the eluent. The highly fluorescent orange band was collected, and chromatograpy repeated. Removal of solvent afforded **5b** (36 mg, 56%) as a black solid. Compound **5b** could be obtained analytically pure either by washing with hot methanol or by high temperature (250°) sublimation, mp 277-278°; ^{1}H nmr (deuteriochloroform): 3.98 (s, 6H, 2(COOC*H*$_3$)), 6.60 (s, 2H, 2(C*H*=C)), 7.18 (dd, 2H, J = 1.6, 8.4 Hz, H-4,11), 7.25 (dd, 2H, J = 7.6, 8.4 Hz, H-3,10), 7.46 (dd, 2H, J = 1.6, 7.6 Hz, H-2,9); ^{13}C nmr (deuteriochloroform): 52.48, 106.84, 118.39, 125.71, 128.44, 130.68, 133.85, 144.38, 147.50, 152.33, 166.91; uv (chloroform/ethanol 2:3) 266.4 nm (log ϵ 4.48), 455.0 nm (sh, log ϵ 4.28), 484.4 nm (log ϵ 4.54), 516.6 nm (log ϵ 4.62); with one drop of concentrated sulfuric acid 264.0 nm (log ϵ 4.46), 411.0 nm (log ϵ 3.62), 547.4 nm (log ϵ 4.33), 583.6 nm (log ϵ 4.42), 629.6 nm (log ϵ 4.23); FAB ms: (+ve) m/z 404 (M+2, 100%), 372 (MH-OCH$_3$, 20%), 344 (MH-COOCH$_3$, 100%).

Anal. Calcd. for $C_{22}H_{14}N_2O_6$: C, 65.67; H, 3.48; N, 6.97. Found: C, 65.34; H, 3.40; N, 6.71.

Acknowledgements.

This work was supported by a grant from the National Health and Medical Research Council of Australia. Mr. G. Corino (Australian Animal Health Laboratories, Geelong) is thanked for providing High Resolution mass spectral data, Mr. J. Korth for translation, Miss G. Martinez for technical assistance and Dr. S. Pyne for helpful discussions.

REFERENCES AND NOTES

[1] J. Gripenberg, *Acta Chem. Scand.*, **12**, 603 (1958).

[2] M. K. Manthey, S. G. Pyne and R. J. W. Truscott, *J. Org. Chem.*, **53**, 1486 (1988).

[3] M. K. Manthey, S. G. Pyne and R. J. W. Truscott, *Tetrahedron.*, **45**, 2803 (1989).

[4] A. Butenandt, J. Keck and G. Neubert, *Liebigs Ann. Chem.*, **602**, 61 (1957).

[5] W. Schafer, I. Geyer and H. Schlude, *Tetrahedron.*, **28**, 3811 (1972).

[6] A. Bolognese, G. Scherillo and W. Schafer, *J. Heterocyclic Chem.*, **23**, 1003 (1986).

[7] A. Bolognese, C. Piscitelli and G. Scherillo, *J. Org. Chem.*, **48**, 3649 (1983).

[8] U. Hollstein, E. Breitmaier and A. Jung, *J. Am. Chem. Soc.*, **96**, 8036 (1974).

[9] M. K. Manthey, S. G. Pyne and R. J. W. Truscott, *Biochim. Biophys. Acta.*, **1034**, 207 (1990).

[10] R. H. Hackman and A. R. Todd, *Biochem. J.*, **55**, 631 (1953). An analogous enzymic oxidation of 3,4-dihydroxybenzoic acid in the presence of aniline according to the procedure in the reference cited also led exclusively to the decarboxylated adduct 4,5-dianilino-1,2-benzoquinone.

[11] E. M. Nicholls and K. G. Rienits, *Int. J. Biochem.*, **2**, 593 (1971).